A Homozygous Deletion Mutation in the Gene Encoding the 180-kDa Bullous Pemphigoid Antigen (BPAG2) in a Family with Generalized Atrophic Benign Epidermolysis Bullosa

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The 180-kDa bullous pemphigoid antigen (BPAG2) is a candidate gene/protein for mutations in some forms of junctional epidermolysis bullosa. In this study, we searched for mutations in BPAG2 in a large Austrian pedigree with generalized atrophic benign epidermolysis bullosa, a distinct nonlethal form of junctional epidermolysis bullosa, using polymerase chain reaction amplification of genomic DNA, heteroduplex analysis of the polymerase chain reaction products, and direct nucleotide sequencing. We identified a homozygous 2-bp deletion within the coding region of BPAG2 in the affected individuals. This mutation results in a frameshift and downstream stop codons on both alleles, predicting an absence of functional protein. These findings illustrate the molecular basis of the skin fragility in this family and attest to the importance of the 180-kDa bullous pemphigoid antigen in the attachment of the epidermis to the underlying dermoepidermal basement membrane. *J Invest Dermatol 106:771–774, 1996*

The hemidesmosome-anchoring filament complex consists of several proteins, including the 230-kDa and the 180-kDa bullous pemphigoid antigens (BPAG1, BPAG2, respectively), the basal keratinocyte $\alpha 6\beta 4$ integrin, and the anchoring filament protein, laminin 5 (Uitto and Christiano, 1992; Yancey, 1995). Immunofluorescence analyses with antibodies to some of these components in patients with junctional epidermolysis bullosa (JEB) have demonstrated altered patterns, implicating several candidate genes for mutations in different forms of this disease (Schofield *et al*, 1990; Fine *et al*, 1991). Specific mutations in JEB individuals have subsequently been delineated in the three genes that encode laminin 5 (LAMA3, LAMB3, and LAMC2) (Aberdam *et al*, 1994; Pulkkinen *et al*, 1994a, 1994b; Uitto *et al*, 1994; Kivirikko *et al*, 1995), and in the $\beta 4$ integrin gene (ITGB4) (Vidal *et al*, 1995).

Generalized atrophic benign epidermolysis bullosa (GABEB) is a subtype of nonlethal JEB, characterized by skin fragility, atrophic alopecia, dysplastic teeth, and nail dystrophy (Hashimoto *et al*, 1976; Schnyder and Anton-Lamprecht, 1979; Hintner and Wolff, 1982). Immunofluorescent antibody staining of the skin of several individuals with GABEB has revealed reduced expression of the 180-kDa bullous pemphigoid antigen (Jonkman *et al*, 1995). In addition, we have recently demonstrated compound heterozygous premature termination codon mutations in the BPAG2 gene in a patient with clinical features of GABEB (McGrath *et al*, 1995a), thus highlighting the molecular heterogeneity of JEB.

In 1982, Hintner and Wolff described a large pedigree with multiple members affected with GABEB (Hintner and Wolff, 1982). Immunofluorescence analysis of basement membrane zone components in this family has recently been reported (Pohla-Gubo *et al*, 1995). The results showed an absence of BPAG2 expression in several affected individuals, implicating BPAG2 as the candidate gene for mutations in this family. The results of mutational analysis in the present study demonstrate that the affected members of this family have a homozygous deletion mutation in BPAG2 and provide evidence for the role of the 180-kDa bullous pemphigoid antigen in adhesion of basal keratinocytes to epidermal basement membrane.

MATERIALS AND METHODS

Clinical The nuclear pedigree subjected to analysis in this study is shown in **Fig 1**. Blood samples for genomic DNA extraction were obtained from eight siblings and their mother; four of the siblings were affected with GABEB, while four individuals as well as their mother were clinically unaffected. An extensive pedigree of this family, encompassing several generations, has previously been published (Hintner and Wolff, 1982). This pedigree raises the possibility of consanguinity in some family members.

Mutation Detection Strategy To search for mutations in the BPAG2 gene, DNA was isolated from peripheral blood by standard methods (Sambrook *et al*, 1989). For amplification of all 56 known exons of the BPAG2 gene, primers based on the flanking intronic sequences were synthesized (Gatalica *et al*, manuscript in preparation). Specifically, amplification of the 390-bp exon containing the homozygous deletion mutation reported in *Results* (nucleotides 3872–4261 in the cDNA; GenBank no. M91669; Giudice *et al*, 1992) utilized the following primers: sense primer,

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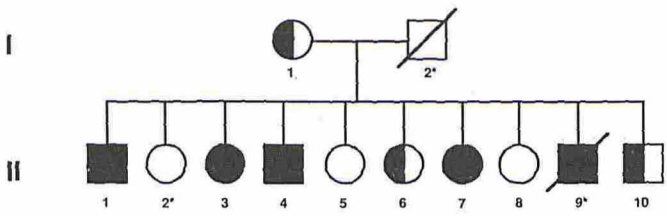

Figure 1. The pedigree of the family with GABEB. Genomic DNA samples were studied from all members apart from those individuals marked by an asterisk. Carrier status is shown with respect to the mutation delineated in this study.

5′-CAAGTCTTTCTCTCCACCGA-3′; anti-sense primer, 5′-CCACAAACAAGAAAGCCAGT-3′. The size of the expected amplification product was 527 bp. For polymerase chain reaction (PCR) amplification, ~250 ng of genomic DNA was used as template in a buffer containing 20 pmol of each primer, 100 mM $MgCl_2$, 2 mM of each of the four nucleotides, and 2.5 U of Taq polymerase (Perkin Elmer Cetus), in a total volume of 50 μl. The amplification conditions were: 94°C for 5 min, followed by 40 cycles of 94°C for 45 s, 55°C for 45 s, 72°C for 45 s, in an OmniGene thermal cycler (Marsh Scientific, Inc.). Following amplification, aliquots of 5 μl were examined on 2% agarose gel electrophoresis, and 3–10 μl of the sample was prepared for heteroduplex analysis (Keen *et al*, 1991) on hydrolink gel according to the manufacturer's recommendations (MDE, J.T. Baker, Inc.). Staining with ethidium bromide was used to visualize the DNA bands. In some cases, the affected individual's amplified DNA was mixed with an equal amount of amplified DNA from an unaffected, unrelated individual, to detect putative homozygous mutations (Ganguly *et al*, 1993). For the region demonstrating heteroduplex formation (see *Results*), the PCR products were sequenced for a heterozygous mutant, a homozygous mutant, and an unrelated control individual using an ABI automated sequencer.

Verification of the Mutation The mutation detected in the BPAG2 gene created a new restriction site for the endonuclease *Nla*III. For verification of the presence of this mutation in genomic DNA, 10 μl of the PCR amplification products from all family members were subjected to *Nla*III restriction enzyme digestion according to the manufacturer's recommendations (New England BioLabs). The digestion products were electrophoresed on 3% agarose gel. In addition to the members of the family studied, 50 unrelated healthy control subjects and 15 individuals with different forms of JEB were analyzed for the possible presence of this mutation by restriction enzyme digestion.

RESULTS

Identification of a homozygous 2-bp deletion within BPAG2 Electrophoretic analysis showed a heteroduplex band, in addition to a homoduplex, in the mother and two unaffected offspring when the PCR amplification product corresponding to a 390-bp exon of BPAG2 was examined **(Fig 2*A*)**. However, only a homoduplex band, similar to that noted in unrelated healthy controls, was detected in the affected individuals. This electrophoretic pattern suggested that the affected individuals were homozygous for a mutation carried by the mother. When amplified DNA from the affected individuals was mixed with amplified DNA isolated from unrelated healthy controls, heteroduplex analysis revealed a heteroduplex band in all four affected individuals. This electrophoretic pattern was identical to that noted with DNA from the mother and the two clinically normal, apparently heterozygous siblings **(Fig 2*A*)**. When DNA from the two other unaffected individuals, who did not initially demonstrate heteroduplex formation, was mixed with control DNA, no heteroduplex was formed. Collectively, these observations suggested that the clinically affected individuals were homozygous for a mutation, the mother and two unaffected siblings were heterozygous carriers of the same mutation, and two unaffected siblings were not carriers of the mutation.

The PCR amplification products derived from the mother (heterozygous carrier), an affected individual, and an unrelated control individual were subjected to direct nucleotide sequencing **(Fig 2*B*)**. The affected individual was homozygous for a 2-bp deletion (TC). This mutation, designated 4003delTC, resulted in a frameshift and premature stop codon (TGA) 86 bp downstream from the site of deletion. The unrelated control individual who did not reveal bands of aberrant mobility on heteroduplex analysis did not show any evidence for this genetic lesion. The mother was shown to be a heterozygous carrier of this mutation, resulting in the frameshift and premature termination codon in one of the alleles while the other allele was normal **(Fig 2*B*)**.

The mutation, 4003delTC, resulted in the generation of a new restriction enzyme site for *Nla*III. Amplification of the exon containing the mutation, together with flanking intronic sequences, resulted in the synthesis of a PCR product of 527 bp which upon digestion with *Nla*III in normal controls yielded five bands, 185, 132, 87, 83, and 40 bp in size **(Fig 2*C*)**. In the presence of the 4003delTC mutation, the 185-bp band was further digest to two fragments, 151 and 32 bp in size. Examination of the PCR product from affected individuals verified that the patients were indeed homozygous for the presence of this mutation since no evidence for the 185-bp band was noted **(Fig 2*C*)**. The results also confirmed that the mother and two of the siblings were heterozygous carriers of the mutation, while two clinically unaffected members of the family, who did not demonstrate heteroduplex formation with or without added control DNA, did not have this mutation. Examination of 50 clinically unaffected unrelated control individuals and 15 patients with different forms of epidermolysis bullosa failed to reveal this mutation in BPAG2, indicating that this genetic alteration is not common in the general gene pool nor does it represent the molecular basis for JEB in the other patients studied thus far.

DISCUSSION

The results of this study have demonstrated a specific 2-bp deletion mutation in the BPAG2 gene, designated as 4003delTC. Four affected individuals in the family were shown to be homozygous for the deletion, while the mother and two clinically unaffected siblings were heterozygous carriers of this mutation. Two of the clinically unaffected individuals were also genotypically normal with respect to this mutation.

The 4003delTC mutation results in a frameshift and formation of a downstream premature termination codon. This mutation probably results in instability of the mRNA transcript, leading to a markedly decreased production of the protein (Urlaub *et al*, 1989; Cheng *et al*, 1990). In support of this interpretation is our recent demonstration of undetectable levels of BPAG2 mRNA in cultured keratinocytes of an individual who was a compound heterozygote for different BPAG2 premature stop codon mutations in a similar region of the gene (McGrath *et al*, 1995a). Likewise, reduced BPAG2 mRNA levels have been demonstrated in several other patients with GABEB, although the specific mutations in the latter cases have not been disclosed (Jonkman *et al*, 1995).

Although it appears that most patients with GABEB have defects in BPAG2 (Jonkman *et al*, 1995), this is not a uniform finding. Recently, a patient with typical features of GABEB was found to have mutations in LAMB3, the gene that encodes the b3 chain of laminin 5 (McGrath *et al*, 1995b). An interesting difference between this patient and the kindred examined in this study was apparent in electron micrographs of the skin. The patient with defective laminin 5 had relatively intact hemidesmosomes (McGrath *et al*, 1995b), whereas affected individuals in this study and the previously reported GABEB patient with BPAG2 mutations, all had hypoplastic hemidesmosomes that were markedly reduced in number (Hintner and Wolff, 1982; McGrath *et al*, 1995a). This suggests that BPAG2 contributes significantly to the structure of the inner and outer hemidesmosomal plaques. It also suggests that these morphological findings may be useful in predicting the underlying candidate gene for a particular patient with GABEB.

Demonstration of a specific genetic lesion underlying the GABEB phenotype in this family has several practical consequences. First, screening of the remaining family members allows determination of the carrier state. Secondly, identification of this mutation now provides the means for DNA-based prenatal diag-

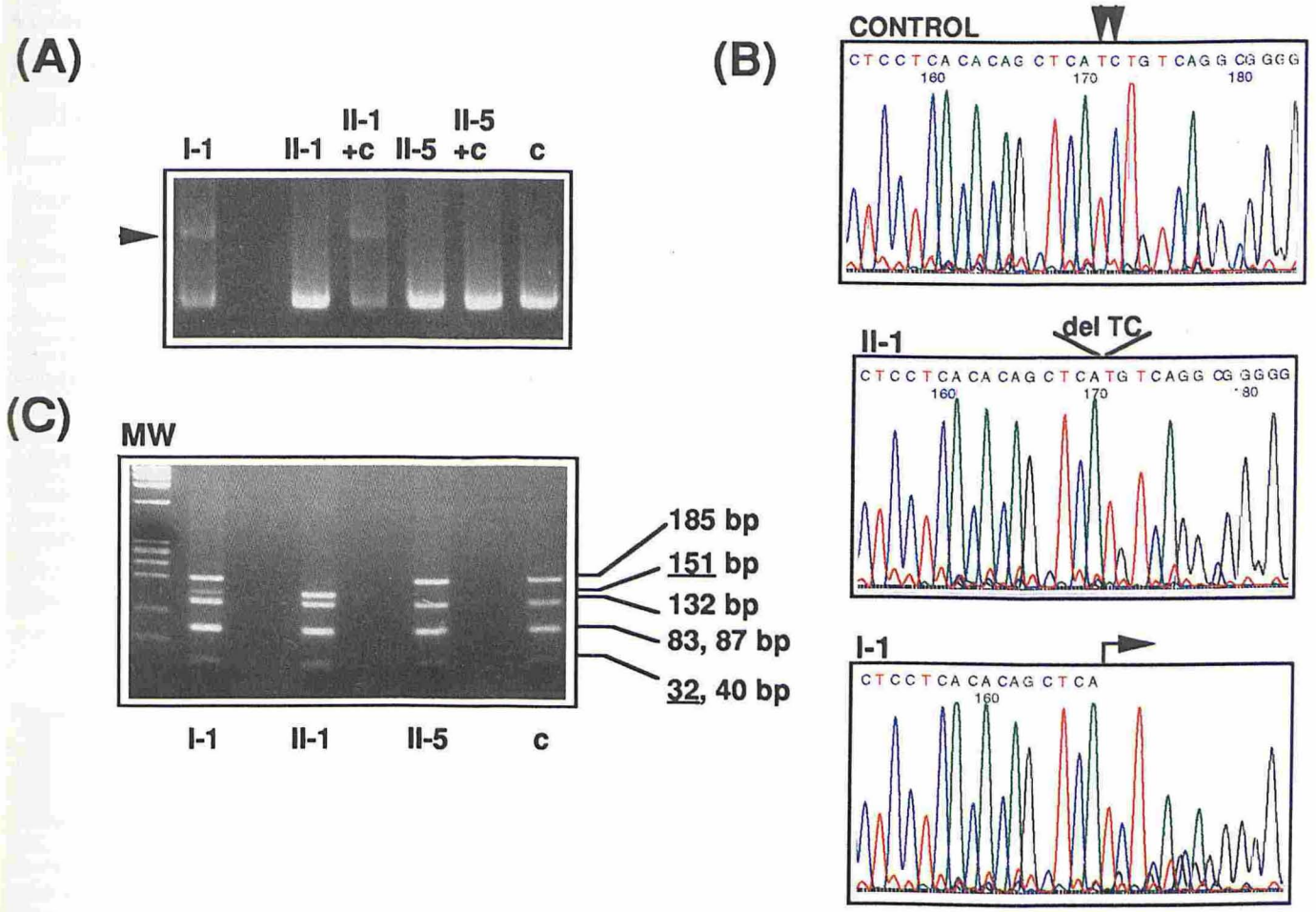

Figure 2. Mutational analysis reveals a homozygous 2-bp deletion in BPAG2 in the GABEB patients in this family. *A*) Heteroduplex analysis of a 527-bp segment of BPAG2 shows a heteroduplex band (➤) in addition to a homoduplex band in the mother (*I-1*), but only homoduplex bands are present in an affected individual (*II-1*) and a sibling (*II-5*). However, when these samples are assessed after mixing with control DNA, an additional heteroduplex band is present in the affected individual (*II-1+c*), but not in the unaffected sibling (*II-5+c*). In the control sample (*c*), only a homoduplex band is detected. *B*) Direct nucleotide sequencing shows the wild-type sequence for part of the PCR product in the top panel. Sequence from an affected individual (*II-1*) reveals a homozygous 2-bp deletion of TC (*middle panel*); these nucleotides are depicted by the two arrowheads in the wild-type sequence. Sequencing of DNA from the mother (*I-1*) demonstrates a frameshift at the site of the arrow (*lower panel*) due to a heterozygous 2-bp deletion on one allele. *C*) Verification of the mutation by restriction endonuclease digestion the enzyme *NIa*III, which creates a new restriction site in the mutant allele. In the control sample (*c*) and the unaffected sibling (*II-5*), the PCR product is cut into digestion products 185, 132, 87, 83, and 40 bp in size. In the affected individual (*II-1*), the 185-bp band is further digested into 151- and 32-bp products. In the mother (*I-1*) both the 185- and 152-bp bands are visible, consistent with a heterozygous carrier of the mutation.

nosis in pregnancies at risk (Christiano and Uitto, 1993). The importance of these considerations is emphasized by the fact that several of the previously affected individuals in this family died soon after birth, according to the original clinical report (Hintner and Wolff, 1982). Finally, knowledge of the specific mutation in BPAG2 gene in this family allows the future development of gene replacement therapy (Taichman, 1994).

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